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# Synthesis and Cytotoxicity Assessment of 2-(4-Fluorophenyl)-*N*-halophenylacetamide Derivatives as Anticancer Agents

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**A B S T R A C T**

Cancer is a group of diseases characterized by uncontrolled proliferation of abnormal cells. Because of the severe side effects and resistance to the traditional chemotherapeutic agents, the necessity for discovery of new anticancer drugs is a crucial topic. In the present study we synthesized new derivatives of 2-(4-Fluorophenyl)-*N*-halophenylacetamide and assessed their cytotoxicity against five cancerous cell lines consisted of PC3 (Prostate cancer), Hela (Cervical cancer), ACHN (Renal cell carcinoma), MCF-7 (Breast cancer) and HL-60 (Promyelocytic leukemia). All compounds demonstrated better cytotoxic activity towards PC3 cell line compared to other cell lines. Compound **2b** showed the best cytotoxic effect in this series ($IC_{50}$ = 102 µM/L). Replacement of butyl chain with phenyl ring led to the increase and improvement of the cytotoxic effects.

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## Introduction

Cancer is a disease in which the control of cell growth and proliferation is lost in one or more cells and finally leading either to a solid tumor such as breast, prostate and etc. cancer or to a liquid cancer like hematological cancers (Leukemia, Lymphoma). It is one of the leading causes of death throughout the world and currently the main therapeutic options involve surgery, chemotherapy and radiotherapy. Chemotherapy involves the use of low molecular weight drugs (small molecules such as methotrexate, cisplatin, doxorubicin…) to selectively destroy tumor cells or at least prevent their proliferation [1]. The survey for identification and discovery of novel chemical structures that can act as more effective and reliable anticancer agents is still a major challenge to medicinal chemists. Despite of the important advances achieved over recent decades in the research and development of various anticancer drugs, current anticancer drugs still have major limitations such as drug resistance, lack of selectivity and unwanted side effects (bone marrow depression, nausea & vomiting…). Hence, there is a strong demand for the discovery and development of effective new cancer therapies devoid of mentioned limitations [2-7].

Despite significant progress achieved in anticancer drugs, the management of malignancies in humans still is a major challenge for current medicine [8-10]. In the recent years, tremendous progress has been made in the war against cancer with the discovery and development of many novel chemotherapeutic agents such as paclitaxel, docetaxel and ixabepilone, as well as small molecule targeted therapies such as imatinib and vorinostat. However, because of toxicity and drug-resistance problems with many current treatments, there is a strong demand for the discovery and development of effective new cancer therapies [11-14]. Since many of the current treatments have problems with toxicity and drug-resistance, there is a strong demand for the discovery and development of effective new cancer therapies.

There are several reports about the anticancer activity of phenylacetamide derivatives (**Fig. 1**) [15-18]. The simple structure and facile synthesis of this structure encouraged us to focus on this scaffold for enhancing and improving its anticancer effects. Hence, replacement of the alkyl(butyl) chain with phenyl residue containing halogen atoms at different positions of the ring was done.

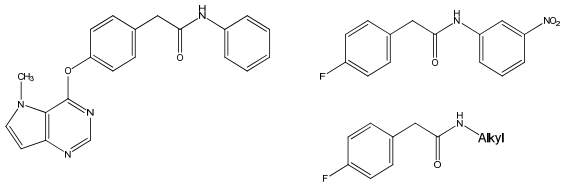



**Fig. 1.** Structures of some phenylacetamide derivatives with anticancer properties.

## Materials and Methods

### *Chemistry*

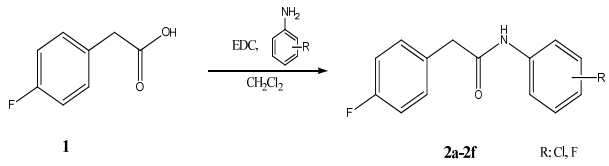
All chemicals including starting materials, reagents and solvents were bought from Merck and Sigma-Aldrich companies as commercial suppliers. Thin layer chromatography was done using TLC sheets of Merck Company. $^1$H NMR spectra were obtained by Brucker 400 MHz and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined using electrothermal melting point analyzer apparatus and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. All cancerous cell lines were purchased from Pasteur Institute of Iran.

### *General procedure for synthesis of compounds* **2a-2f**

All compounds **2a-2f** were synthesized according to the **scheme 1**. In a flat bottom flask 200 mg (1.2 mmol) of *p*-Fluorophenylacetic acid and 248 mg (1.2 mmol) *N*-ethyl-*N*-dimethylaminopropyl carbodiimide (EDC) were stirred in 10 ml of dichloromethane ($CH_2Cl_2$) for 30 minutes. Then, equivalent amount of appropriate aniline derivative was added and stirring condition was continued for 24 hours. The progress of the reaction was checked using thin layer chromatography (TLC). After completion, the solvent was evaporated under reduced pressure and equal portions of water and ethyl acetate (25 ml) were added to the residue. The aqueous phase was removed and the organic phase was treated two times by sodium bicarbonate 10% solution, sulfuric acid

5% and brine. Anhydrous sodium sulfate was used for drying and then dichloromethane was evaporated using rotary evaporator apparatus. The obtained product was triturated by diethyl ether and *n*-hexane for eliminating the impurities [19].




**Scheme 1.** Synthesis of compounds **2a-2f.**

***N-(2-Chlorophenyl)-2-(4-fluorophenyl)acetamide* (2a)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.68 (s, $CH_2$), 7.13-7.20 (t, *J* = 8Hz, *p*-Fluorophenyl), 7.30 (t, *J* = 8Hz, *o*-Chlorophenyl), 7.37 (t, *J* = 8Hz, *p*-Fluorophenyl), 7.48 (d, *J* = 8Hz, *o*-Chlorophenyl), 7.55 (t, *J* = 8Hz, *o*-Chlorophenyl), 7.66 (d, *J* = 12Hz, *o*-Chlorophenyl), 9.73 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3255 (NH, Stretch), 1658 (C=O, Stretch), 1531 (NH, Bend), 1500, 1490 (C=C, Aromatic), 1438, 1222, 1186, 1157, 1136, 759, 742. MS (*m/z*, %): 265 ($M^+$+2, 10), 263 ($M^+$, 30), 228 (75), 127 (90), 109 (100), 83 (40), 63 (20).

***N-(3-Chlorophenyl)-2-(4-fluorophenyl)acetamide* (2b)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.65 (s, $CH_2$), 7.09 (d, *J* = 8Hz, *m*-Chlorophenyl), 7.15 (t, *J* = 8Hz, *m*-Chlorophenyl), 7.30-7.37 (m, aromatic), 7.44 (d, *J* = 8Hz, *m*-Chlorophenyl), 7.81 (s, *m*-Chlorophenyl), 10.36 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3250 (NH, Stretch), 1658 (C=O, Stretch), 1600 (C=C, Aromatic), 1544 (NH, Bend), 1508, 1481 (C=C, Aromatic), 1411, 1400, 1350, 1292, 1157, 894, 866, 773. MS (*m/z*, %): 265 ($M^+$+2, 15), 263 ($M^+$, 50), 154 (10), 127 (80), 109 (100), 83 (30), 63 (15).

***N-(4-Chlorophenyl)-2-(4-fluorophenyl)acetamide* (2c)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.63 (s, $CH_2$), 7.14 (t, *J* = 8Hz, *p*-Fluorophenyl), 7.34 (m, aromatic), 7.61 (d, *J* = 8Hz, *p*-Chlorophenyl), 10.34 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3282 (NH, Stertch), 1660 (C=O, Stretch), 1591, 1525, 1500, 1489, 1396, 1300, 1091, 817. MS (*m/z*, %): 265 ($M^+$+1, 5), 263 ($M^+$, 15), 127 (90), 109 (100), 99 (30), 83 (40), 63 (20).

***N-(2-Fluorophenyl)-2-(4-fluorophenyl)acetamide* (2d)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.72 (s, $CH_2$), 7.15 (t, *J* = 8Hz, *p*-Fluorophenyl-$CH_2$-), 7.25 (m, *o*-Fluorophenyl-NH-), 7.36 (t, *J* = 8Hz, *p*-Fluorophenyl-$CH_2$-), 7.86 (m, *o*-Fluorophenyl-NH-), 9.96 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3255 (NH, Stretch), 1670 (C=O, Stretch), 1614 (C=C, Aromatic), 1535 (NH, Bend), 1500, 1490 (C=C, Aromatic), 1454, 1346, 1291, 1217, 1188, 754. MS (*m/z*, %): 248 ($M^+$+1, 10), 247 ($M^+$, 60), 109 (100), 83 (55).

***N-(3-Fluorophenyl)-2-(4-fluorophenyl)acetamide* (2e)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.64 (s, -$CH_2$-), 6.85 (t, *J* = 12Hz, aromatic), 7.14 (t, *J* = 8Hz, aromatic), 7.29 (t, *J* = 8Hz, aromatic), 7.23-7.37 (m, aromatic), 7.68 (d, *J* = 12Hz, aromatic), 10.41 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3261 (NH, Stretch), 1662 (C=O, Aromatic), 1598 (C=C, Aromatic), 1552 (NH, Bend), 1508, 1500, 1425, 1222, 1199, 1155, 775. MS (*m/z*, %): 248 ($M^+$+1, 10), 247 ($M^+$, 75), 136 (30), 109 (100), 83 (30), 57 (10).

***N,2-bis(4-fluorophenyl)acetamide* (2f)**

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 3.62 (s, $CH_2$), 7.11 (t, *J* = 4Hz, *p*-Fluorophenyl-NH), 7.12 (d, *J* = 2.8Hz, *p*-Fluorophenyl-NH), 7.14 (d, *J* = 2.8Hz, *p*-Fluorophenyl-NH), 7.16 (t, *J* = 4Hz, *p*-Fluorophenyl-NH), 7.35 (t, *J* = 8Hz, *p*-Fluorophenyl-$CH_2$-), 7.59 (t, *J* = 8Hz, *p*-Fluorophenyl-$CH_2$-), 10.23 (brs, NH). IR (KBr, $cm^{-1}$) $\bar{\upsilon}$: 3251 (NH, Stretch), 1649 (C=O, Stretch), 1602 (C=C, Aromatic), 1500 (NH, Bend), 1406, 1220, 1153, 825. MS (*m/z*, %): 248 ($M^+$+1, 5), 247 ($M^+$, 35), 137 (10), 109 (100), 83 (80), 57 (20).

## *MTS assay*

The MTS assay is based on the reduction by mitochondrial dehydrogenase in metabolically active cells of the novel tetrazolium compound, MTS, to the water-soluble formazan that absorbs at 490 nm. PC3, MCF7, ACHN, Hela and HL-60 cells were seeded in

each well of a 96-microwell plate and treated with various concentrations of the compound(s). After incubation for 48 h, Cell Titer 96 Aqueous One Solution Reagent (Promega, Madison, WI), which is composed of the novel tetrazolium compound MTS and an electron coupling reagent phenazine ethosulfate (PES, a redox intermediary), was added to each well according to the manufacturer's instructions. After 3 h, cell viability was determined by measuring the absorbance at 490 nm using an ELISA microplate reader (Awareness, Palm City, FL). The cytotoxicity of the compound(s) was presented as mean of 3 independent experiments with 3 replicates for each compound(s) concentration [20].

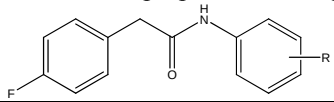
## Results and Discussion

### *Chemistry*

All intended compounds **2a-2f** were synthesized according to the **scheme 1** with an average yield (40-65 %) (**Table 1**). Direct coupling of 4-Flurophenylacetic acid (**1**) with various aniline derivatives was carried out using *N*-ethyl-*N*-dimethylaminopropyl carbodiimide (EDC) in acetonitrile solvent. Hydroxybenzotriazole (HOBt) was also added to the reaction medium to prevent the formation of side products.

Melting points of compounds **2a-2f** were measured using open capillary tubes by melting point analyzer as listed in **Table 1**. A range of 118-170 $^0$C of melting points was obtained in this series. In chlorinated derivatives (**2a-2c**) as well as fluorinated derivatives (**2d-2f**), compounds with *para* substitution exhibited a higher melting point compared to other types (*ortho* and *meta*).

**Table 1.** Physicochemical properties of compounds **2a-2f**.




| Compounds | R | Closed Formula | MW (g/mol) | Yield (%) | mp ($^0$C) | Appearance |
|---|---|---|---|---|---|---|
| 2a | *o*-Cl | $C_{14}H_{11}ClFNO$ | 263 | 65 | 118 | White powder |
| 2b | *m*-Cl | $C_{14}H_{11}ClFNO$ | 263 | 53 | 130 | White powder |
| 2c | *p*-Cl | $C_{14}H_{11}ClFNO$ | 263 | 40 | 170 | White powder |
| 2d | *o*-F | $C_{14}H_{11}F_2NO$ | 247 | 45 | 121 | White powder |
| 2e | *m*-F | $C_{14}H_{11}F_2NO$ | 247 | 42 | 118 | White powder |
| 2f | *p*-F | $C_{14}H_{11}F_2NO$ | 247 | 53 | 147 | White powder |

Spectroscopic methods such as $^1$H NMR, IR and MS were applied for characterization of the prepared compounds **2a-2f**. All compounds were dissolved in deutrated dimethylsulfoxide (DMSO-$d_6$) for acquisition of NMR spectra. Broad singlet peak of the NH proton is a good sign for formation of the amidic bond in this series. Potassium bromide (KBr) disk was used to obtain the infra red (IR) spectrum related to each compound. The peak related to the carbonyl group appeared in <1700 cm$^{-1}$ is a sign of the amidic carbonyl group in IR spectrum and it is a confirmation for the formation of the amidic moiety in these compounds. $M^+$+2 peak of the chlorine substituted derivatives confirms the presence of chlorine atom in these derivatives.

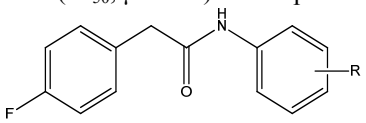
### *Cytotoxicity assessment*

According to **Table 2**, all compounds **2a-2f** were examined against five cancerous cell lines consisted of PC3, AHCN, Hela, MCF-7 and HL-60. All Compounds showed a higher cytotoxic activity toward PC3 cell line. Compound **2b** with *meta*-chloro substituent was the best compound in this series. Substitution of the chlorine at position 2 of the phenyl ring led to the lowest cytotoxic effect for this moiety. Substitution of chlorine at position 4(*para*) of the phenyl ring caused a moderate activity for

compound **2c** compared to other positions. Replacement of chlorine atom with fluorine led to the similar trend of cytotoxic activity. It means that like compound **2a-2c**, compound **2e** with *meta* substitution of fluorine demonstrated a higher potency in comparison with compounds **2d** and **2f** with fluorine substitution at other positions of the phenyl ring. Besides, compound **2d** with *ortho* substitution like *ortho* chlorine exhibited the lowest potency toward PC3 cell line. Finally, all compounds **2a-2f** did not showed higher potency than imatinib in all cell lines.

**Table 2.** Cytotoxicity results ($IC_{50}$, µmol/L) of compounds **2a-2f** after MTS assay.




| Compounds | R | PC3 | ACHN | Hela | MCF-7 | HL-60 |
|---|---|---|---|---|---|---|
| 2a | *o*-Cl | 193±3.21 | >250 | >250 | >250 | 248±1.2 |
| 2b | *m*-Cl | 102±2.02 | >250 | >250 | >221 | 217±0.76 |
| 2c | *p*-Cl | 158±0.21 | >250 | >250 | >250 | 246±0.04 |
| 2d | *o*-F | 175±0.18 | >250 | >250 | >250 | 200±2.2 |
| 2e | *m*-F | 121±0.08 | >250 | >250 | >250 | 197±1.24 |
| 2f | *p*-F | 152±2.36 | >250 | >250 | >250 | 218±0.09 |
| Imatinib | | 40±0.06 | ND* | ND | 68±0.03 | 98±1.74 |

- Not Determined

## Conclusion

Insertion of the phenyl ring instead of butyl chain into the structure of *p*-Fluorophenylacetamides improved the cytotoxic activity. All synthesized compounds showed higher cytotoxic effect (µM range) than lead compound **A** (Mm range) (**Fig. 1**).

## Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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## References

[1] Nussbaumer S, Bonnabry P, Veuthey J, Fleury-Souverain S. Analysis of anticancer drugs: A review. Talanta. 2011;85:2265–2289.

[2] Kaushik G, Kaushik T, Khanduja S, Pathak CM, Khanduja K. Cigarette smoke condensate promotes cell proliferation through disturbance in cellular redox homeostasis of transformed lung epithelial type-II cells. Cancer Lett. 2008;270:120–131.

[3] Albreht T, Mckee M, Alexe DM, Coleman MP. Martin-Moreno JM. Making progress against cancer in Europe in 2008. Eur J cancer. 2008;44:1451–1456.

[4] Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol. 2007;18:581–592.

[5] Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer Statistics, 2006. Cancer J Clin. 2006;56:106–130.

[6] Bhuva H.A, Kini SJ. Synthesis, anticancer activity and docking of some substituted benzothiazoles as tyrosine kinase inhibitors. J Mol Graph Model. 2010;29:32–37.

 

[7] Madhusudan S, Trivadi S, Ganesan TS. Tyrosine kinase inhibitors in cancer therapy. Clinnical Biochem. 2004;37:618–635.
[8] Rzeski W, Matysiakb J, Kandefer-Szerszen M. Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound. Bioorg Med Chem. 2007;15:3201–3207.
[9] Kemnitzer W, Kuemmerle J, Zhang HZ, Kasibhatla S, Tseng B, Drewe J, Cai SX. Discovery of 3-aryl-5-aryl-1,2,4-oxadiazoles as a new series of apoptosis inducers. 2. Identification of more aqueous soluble analogs as potential anticancer agents. Bioorg Med Chem Lett. 2009;15:4410–4415.
[10] Kemnitzer W, Kuemmerle J, Jiang S, Zhang HZ, Sirisoma N, Kasibhatla S, et al. Discovery of 1-benzoyl-3-cyanopyrrolo[1,2-a]quinolines as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. Part 1: Structure-activity relationships of the 1- and 3-positions. Bioorg Med Chem Lett. 2008;(18):6259–6264.
[11] Bortner CD, Cidlowski JA. A Necessary Role for Cell Shrinkage in Apoptosis. Biochem Pharmacol. 1998;56:1549–1559.
[12] Sirisoma N, Pervin A, Drewe J, Tseng B, Cai SX. Discovery of substituted N'-(2-oxoindolin-3-ylidene)benzohydrazides as new apoptosis inducers using a cell- and caspase-based HTS assay. Bioorg Med Chem Lett. 2009;19:2710–2713.
[13] Glamoclija V, Vilovic K, Saraga-Babic M, Baranovic A, Sapunar D. Apoptosis and active caspase-3 expression in human granulosa cells. Fertil Steril. 2005;83:426–431.
[14] Robertson GS, Crocker SJ, Nicholson DW, Schulz JB. Neuroprotection by the Inhibition of Apoptosis. Brain Pathol. 2000;10:283–292.
[15] Chan HC, Kuo SC, Liu SC, Liu CH, Hsu SL. 4-Fluoro-N-butylphenylacetamide: a synthetic phenylacetate derivative that upregulates Bcl-XS, activates caspase cascade and induces apoptosis in human squamous lung cancer CH27 cells. Cancer Lett. 2002;18:212–221.
[16] Liu CH, Huang LJ, Kuo SC, Lee TH, Tsai KJ, Chan HC. 4-Fluoro-N-butylphenylacetamide (H6) inhibits cell growth via cell-cycle arrest and apoptosis in human cervical cancer cells. Bioorg Med Chem. 2009;17:42–48.
[17] Oguro Y, Miyamoto N, Okada K, Takagi T, Iwata H, Awazu Y. Design, synthesis, and evaluation of 5-methyl-4-phenoxy-5H-pyrrolo-[3,2-d]pyrimidine derivatives: Novel VEGFR2 kinase inhibitors binding to inactive kinase conformation. Bioorg Med Chem. 2010;18:7260–7273.
[18] Aliabadi A, Andisheh S, Tyarani-Najaran Z, Tayarani-Najaran M, 2-(4-Fluorophenyl)-N-phenylacetamide derivatives as anticancer agents: synthesis and in vitro cytotoxicity evaluation. Iran J Pharm Res. 2012; in press.
[19] Aliabadi A, Shamsa F, Ostad SN, Emami S, Shafiee A, Davoodi J, et al. Synthesis and biological evaluation of 2-Phenylthiazole-4-carboxamide derivatives as anticancer agents. Eur J Med Chem. 2010;11:5384–5389.
[20] Malich G, Markovic B, Winder C. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. Toxicol. 1997;124:179–192.

 